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CHANGES IN COMPOSITION OF SKELETAL MUSCLES DURING GROWTH IN THE RAT

by

YUH-LIN YU

A THESIS
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FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Changes in Composition of Skeletal Muscles During Growth in the Rat" submitted by Yuh-Lin Yu, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

Two experiments were conducted with rats to investigate the effects of different planes of nutrition on changes in the chemical composition of the whole body and of skeletal muscles. Experiment I was a preliminary study on the normal growth of rats aging from 1 to 62 days. In experiment II 56 rats (27 males nnd 29 females) were divided into control, low plane and realimented groups in order to demonstrate the effects of different planes of nutrition on the growth processes. The experiment started when the rats were 26 days of age and ended when they were 100 days of age. Low-plane rats received a ration consisting of the stock ration diluted by 50 percent with cellulose. One group of low-plane rats was subsequently realimented with the undiluted stock ration after the 5th week of the experimental period. In both experiments rats were killed at intervals for chemical analysis.

The chemical parameters measured included total water, nitrogen of the whole body and of the muscles (quadriceps and hind limb muscles), and the specific protein fractions and DNA in hind limb muscles. The results indicated that skeletal muscles had a higher growth rate than the body as a whole and that restricted feeding tended to produce a higher muscle/body weight ratio. Both whole body and muscle demonstrated an increasing N/H_2O ratio with maturity. The muscle protein fractions showed a differential pattern in which the myofibrillar protein increased at a faster rate and connective tissue at a lower rate relative to the sarcoplasmic protein. There was a decrease in DNA relative to muscle dry weight as body weight increased. Restricted feeding resulted in about a 50 percent decrease in body weight relative to that of controls of equal age with an apparent slowing in the rate of physiological aging

with respect to chronological time. On a dry weight basis, there seemed to be a similar pattern of the growth process in both controls and low plane rats in terms of changes in N/H_2O, N/DNA and myofibrillar protein N/DNA. By these same parameters, realimentation of low plane rats essentially resulted in an acceleration of physiological aging to a rate comparable to that observed in the controls.

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INTRODUCTION

Control of meat quality requires a basic understanding of the nature of growth and development in the muscle tissue. Growth is a complex process which involves a very large number of factors, both genetic and environmental. The effects on growth of such factors as sex, age, plane of nutrition, physical activity and anatomical location of particular body components have been quite extensively demonstrated on a chemical basis in terms of water, nitrogen and fat. Recently investigations at the biochemical level have begun to receive attention. The present research was undertaken to study some aspects of the fundamental process of muscle growth and carcass quality in growing animals. The rat was used as an experimental animal in the present study. It was hoped to establish techniques for subsequent investigations of a similar nature on animals used for meat production. The present experiments were designed to investigate the following aspects of growth and development in whole rats and in their skeletal muscles.

a) The compositional changes that occurred in the whole body of growing rats with emphasis on the nitrogen/water relationship.

b) The growth rate in skeletal muscles and the extent to which changes in nitrogen/water ratio that occurred in skeletal muscles followed changes in nitrogen/water ratio of the whole body.

c) The changes in the proportions of the various muscle protein fractions and the measurement of the DNA content of skeletal muscles. The relationship of whole muscle nitrogen to DNA and of myofibrillar protein nitrogen to DNA, were taken

to indicate the accumulation of total muscle protein and of myofibrillar protein respectively in a cell unit.

d) The effect of restricted growth rate resulting from the dilution of the ration with non-digestible materials and the effect of subsequent realimentation on the parameters listed above.

REVIEW OF LITERATURE

Continuous change, which is a fundamental characteristic of living organisms, is brought about by a multitude of individual processes associated with growth, development and aging. Each individual process is further influenced by the nature of the environment to which the animal is exposed; for example, the quality and availability of food, the physical environment in which the animal is kept and the amount of activity which it performs.

A survey of literature has been carried out on experiments designed to measure, on one hand, the growth rate and N/H_2O ratio in skeletal muscles in relation to changes in N/H_2O in the whole body during growth, and on the other changes in the protein distributions and DNA content of skeletal muscles in growing animals of various species on different planes of nutrition.

I. Changes in Water and Nitrogen in the Whole Body During Growth

It is well known that the bodies of young animals contain a higher proportion of water than do adults of the same species (Moulton, 1923; Pace and Rathburn, 1947; Bailey, Kitts and Wood, 1960; Wood and Groves, 1965; Groves and Wood, 1965). Lowry and Hastings (1952) explained the relative decrease in body water content that occurs with growth in terms of a relative increase in the number of cells per unit of body size accompanying growth of the tissues and a resultant relative decrease in the amount of the extracellular fluid.

After water, protein makes up the largest contribution to the fat free body weight. Studies have shown that the proportion of water falls with growth and aging and the concentrations of nitrogenous

constituents rise; there is thus an increase in the ratio of N/H O with increasing body weight and age, though the rate of increase declines with age (Keys and Brozek, 1953; McCance and Dickerson, 1956; Bailey et al, 1960; Wood and Groves, 1965).

II. Growth Rate and Changes in Water, Nitrogen and Protein Distributions in Skeletal Muscles During Growth

The growth rate of skeletal muscle has been extensively studied. Hammond (1932) indicated that different muscles develop at different rates as animals grew from birth to maturity. McMeekan (1940) and Pálsson and Vergés (1953) provided further evidence of differential muscle growth. More recently, the differential development of the chemical components of muscle has also been demonstrated (Lawrie, 1961a, b; Lawrie and Cathern, 1964).

In general, skeletal muscles grow at approximately the same rate during the embryonic stage and for a period of time after birth. This period varies between species. At a later age different muscles not only show different growth rates but a relatively faster rate of growth is noted in all muscles (McMeekan, 1940).

As had already been indicated for the whole body a considerable dehydration of skeletal muscle tissue occurs during growth in very young animals (Needham, 1931; Nikitin, 1936; McMeekan, 1940; Horvath, 1946; McCance and Widdowson, 1956; Dickerson and Widdowson, 1960; Lawrie, 1961b). At the same time there is an absolute and relative increase in the protein content of muscle, indicating the growth of the muscle cell mass (Needham, 1931; McCance and Widdowson, 1956; Dickerson and Widdowson, 1960; Lawrie, 1961a,b).

Robinson (1952a) described a modified version of the method of

Herrmann and Nicholas (1948) for the fractionation of muscular proteins. This method enables a complete separation of extracellular or connective tissue protein from intracellular protein to be achieved. The intracellular protein can moreover, be fractionated further into myofibrillar (contractile) and sarcoplasmic proteins, in conformity with histological observations of muscle structure.

Robinson (1952a) reported that the percentage of total nitrogen in the muscles of chicks increased brom birth and levelled off at about 100 gm body weight or at about 2 weeks of age. However, he showed also that as the chicks grew from birth to maturity, myofibrillar proteins came to represent an increasingly larger fraction of the total protein, although, from about 2 weeks after hatching, the rate of this increase declined; the sarcoplasmic protein, therefore, represented a steadily decreasing percentage of total protein though they also showed a steadily increasing portion if expressed as a percentage of fresh weight. Herrmann (1952) indicated that in chick muscle the actomyosin content rose steeply during the first month. Sharp increases in contractile and sarcoplasmic proteins of pig and human muscles have been observed during the early period of development (Dickerson and Widdowson, 1960). Helander (1957) reported comparable values from cattle episodically from 5 days after birth to 14 years of age. Lawrie (1961b) found increments in both myofibrillar and sarcoplasmic proteins in bovine muscles during development from birth to maturity; the relative changes in these components, however, were different for each muscle. The growth curve of sarcoplasmic and myofibrillar proteins has also been studied in growing rats aging from 43 to 153 days after birth (Gordon,Kowalski and Fritts, 1966).

III. Changes in DNA Content and Its Relationship to Protein Growth in the Skeletal Muscles

Herrmann and Nicholas (1949) reported a sharp decrease in the DNA content of skeletal muscles of rats from the embryonic stage through weaning. Herrmann (1952) also indicated similar changes in DNA content in growing chicks. A large difference in muscle DNA content between 3-week old chicks anc chick embryos was reported by Robinson (1952b). Herrmann, White and Cooper (1957) studied the growth of leg musculature in developing chicks using DNA content as an index of cell proliferation and the accumulation of specific proteins such as actomyosin and collagen as a unit of cell size. Konisberg (1958) indicated that the ratio of protein nitrogen to DNA content can be used as an expression of the nucleocytoplasmic unit. It has been indicated that the weight of muscle increased more rapidly than the DNA content in the muscle (Enesco, 1960, 1961; Enesco and Leblond, 1962). Gordon et al (1966) by determination of the DNA content and the structural proteins in the quadriceps muscles of the growing rat demonstrated that growth in this muscle during the first 90 days was associated both with proliferation of nuclei and with hypertrophy of individual muscle cells, but occurred with hypertrophy alone in the following 50 days.

IV. Effects of Plane of Nutrition on the Above Parameters

Different planes of nutrition also exhibit differential influences on the growth rate of individual skeletal muscle (McMeekan, 1940; Pálsson and Vergés, 1952). When the growth of young rats was retarded for 2 weeks by feeding them a low protein diet and the animals were subsequently fed an adequate ration, no apparent changes in the total nitrogen content of the whole body or of muscle tissue were noted

(Widdowson and McCance, 1957). Stainner (1957) obtained similar results from a similar experiment on adult rats. Lowry et al (1942) showed a higher water content in the skeletal muscles of rats which had been underfed for about 2 years and were one third of normal size. Dickerson and McCance (1960) showed that in the chicken, prolonged severe underfeeding if begun early in life, gave rise to an increase in water and a decrease in the total nitrogen content of muscle. Moreover, the growth or hypertrophy of individual muscle cells (intracellular proteins) was retarded to a greater extent than was the growth of the connective tissues. The contents of myofibrillar and sarcoplasmic proteins of chronically undernourished animals remained similar to those of the very young normal control animals. Rehabilitation was able, however, to restore both muscle growth and chemical composition to normal adult values. Similar results were obtained in the pig (Widdowson, Dickerson and McCance, 1960). Cabak, Dickerson and Widdowson (1963) showed that when young rats were kept for 26 days on a low protein ration they lost 37% of their original body weight; the skeletal muscles, however, lost relatively more weight than the body as a whole. This was shown to be because the intracellular proteins decreased whereas the extracellular proteins tended to increase.

Mendes and Waterlow (1958) first demonstrated that the ratio of nitrogen to DNA was greatly reduced in skeletal muscles of young rats whose growth was completely arrested by a protein deficient diet. This reduced N/DNA ratio was due mainly to a decrease in intracellular proteins, since the connective tissue continued to increase in amount throughout the depletion period. Montgomery, Dickerson and McCance (1964) reported that in birds the ratio of intracellular protein N to

DNAP, which was used as a measure of the size of the cell unit, was much lower for undernourished chickens, and that subsequent refeeding essentially restored this ratio to normal.

EXPERIMENTAL

I. Experimental Design

A. Experiment I

A preliminary study was undertaken to observe the growth rate and the changes in the chemical composition of skeletal muscle during normal growth. Thirty rats aging from 1 to 62 days were used for analysis. The stock ration fed was the same as that received by the control group in Experiment II. The composition of this ration is indicated in Appendix I.

B. Experiment II

Fifty-six male and female rats were used to study the effect of different planes of nutrition on body and muscle growth. The experiment started when the rats were 26±1 days of age and ended at 100±4 days. At the beginning, animals were divided into 2 groups--control and low plane. Rats in the control group received the stock ration. Low plane rats received the stock ration diluted with cellulose to limit both energy and nitrogen intake. Both groups of rats were allowed to feed ad libitum. Four rats were killed at the beginning forming the initial control for further body composition comparisons. Two to four of each group were killed at weekly intervals during the first 6 weeks. Starting from the 5th week of the experiment, 15 rats (7 males and 8 females) of the low plane group were fed with the same stock ration as the control. These rats formed the realimented group. The experiment was terminated in the 11th week when all the remaining animals were killed.

II. Ration

The stock ration contained 21.2% of crude protein. The detailed

composition of this ration is indicated in the Appendix I. The diet for the low plane rats was made up from equal parts by weight of the stock ration and powdered cellulose (Solkafloc). Clean drinking water was available to all animals throughout the experiments.

III. Killing and Dissection of Animals

The rats were first weighed and then killed with ether. The dissection of the rats was carried out at $0^{o}C$ to $4^{o}C$ in order to minimize post-mortem changes. In experiment II, separate determinations were made on the combined muscles of one whole hind limb and on the quadriceps muscles of the other limb, while in experiment I, only the combined muscles of one hind limb were removed. The tendons, visible fat and nerves were discarded before weighing the muscles which were then minced finely with scissors and stored at $-15^{o}C$ in a sealed bottle. The remaining carcass was weighed immediately and directly transferred to a drying oven for the determination of the water content.

IV. Chemical Analysis

A. Water

The carcass and samples of the collected muscles were dried to constant weight in an oven at $100^{o}C$ for the determination of water. The water content of the carcass was corrected to account for the water contained in the muscle samples.

B. Total Nitrogen

Total nitrogen in the whole carcass and muscles was determined by the macro- and micro- Kjeldahl methods respectively. The nitrogen content of the whole carcass was corrected for the nitrogen in the removed muscles.

C. Fractionation of Muscle Proteins

The muscle proteins were separated from fresh muscle using both the method described by Robinson (1952) and a slightly modified technique reported by Steward (1955). The nitrogen determination for each fraction was carried out by the micro-Kjeldahl method. The procedure for fractionation of the muscle protein is listed in the Appendix II.

D. DNA

The methods used for DNA determination were based on a combination of the method of Schmidt and Thannhauser (1945) and the method of Schneider (1945) as used by Logan, Mannell and Rossiter (1952a,b). The phosphorus was determined by the method of Allen (1940). The standard DNA was a commercial preparation of calf sodium thymonucleate*. The procedures used are outlined in the Appendix II.

---

*B.D.H. Laboratory chemicals

RESULTS

EXPERIMENT I

I. The Effect of Growth on the Contents of Water and Nitrogen in the Whole Body

The results of experiment I showed that the relative decrease that occurred in water content of the body during development was accompanied by a relative increase in the amount of total nitrogen. The water content decreased from 82% at birth to 70% of the whole body at 2 months of age.

There were no significant changes in the nitrogen content expressed as a percentage of dry body weight. The results are indicated in Appendix III. There was, however, an increase in the ratio of nitrogen relative to water as age and body weight increased (Fig. 6). A rapid increase in N/H_2O occurred between birth and the weaning age of 21 days.

II. The Effect of Growth on Water, Total Nitrogen, and the Different Protein Fractions of Skeletal Muscle

The water content of skeletal muscles decreased from 87% at birth to 77% at 2 months of age (Appendix III). This followed the pattern of the whole body. There was a rise in nitrogen relative to the dry weight of muscles during growth; this rose from 11% at birth to 13.5% after about 1 month of life (Appendix III). Again, as in the whole body, N/H_2O ratio in the skeletal muscles increased at a more rapid rate before weaning than afterwards.

Quantitative changes in the muscle protein fractions as the muscles increased in weight are shown in Fig. 1. A steady and marked increase of intracellular protein was noted with development while there was only a slight increase in the extracellular protein, assumed to be primarily collagen. Among the intracellular proteins, the myofibrillar

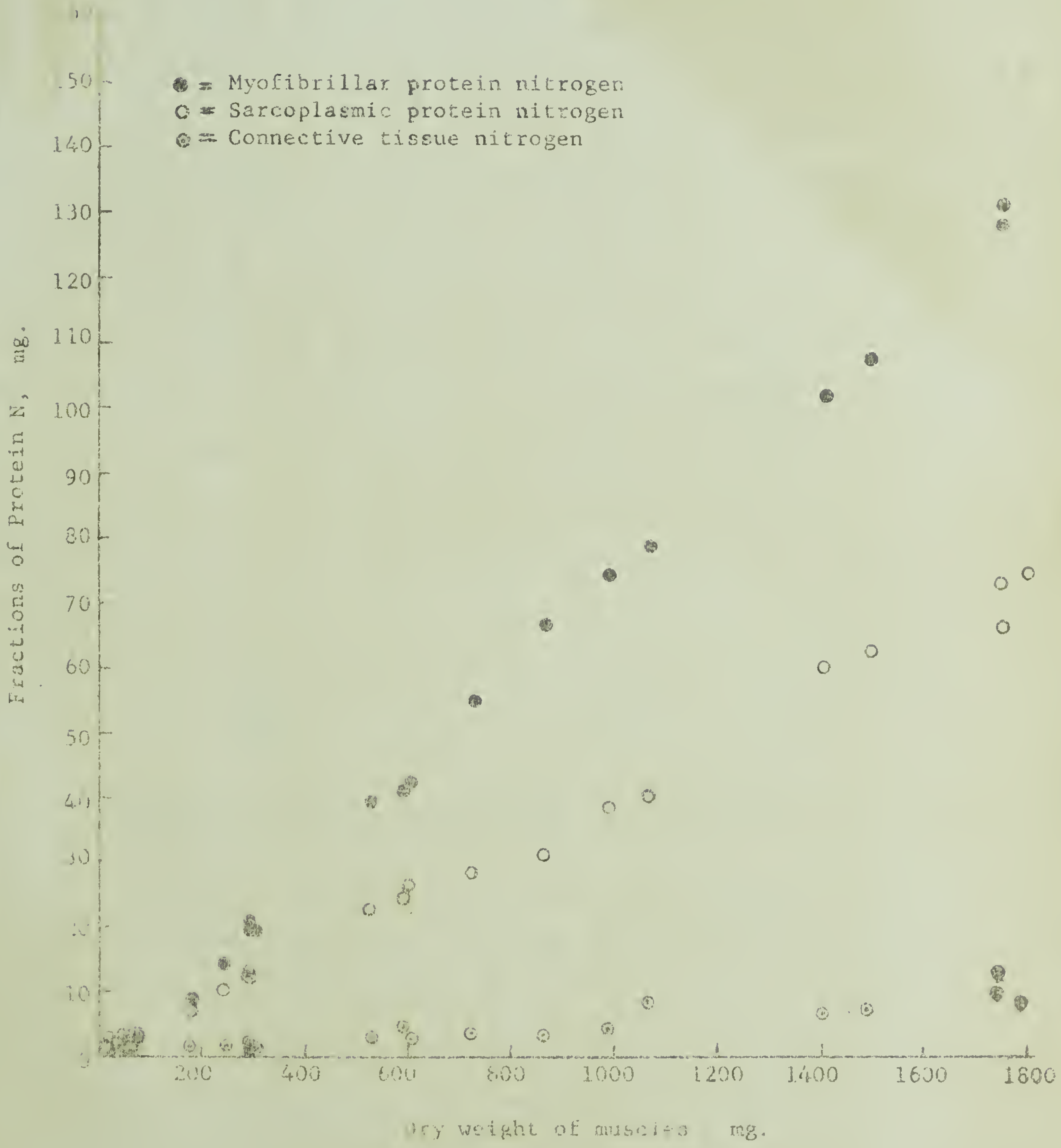

Fig. 1. Changes in the protein fraction of hind limb muscle as a function of dry muscle weight, Experiment 1.

protein increased at almost twice the rate of the sarcoplasmic protein after weaning. In Fig. 2 the protein fractions are expressed as a percentage of total nitrogen. Myofibrillar protein nitrogen showed a sharp increase during the first 2 weeks after birth. At about 4 weeks it reached a steady state, representing a considerably larger portion of total nitrogen than at birth. Myofibrillar protein nitrogen accounted for about 23% of the total nitrogen at birth and rapidly increased to 55% of the total nitrogen at about 4 weeks of age. At birth the sarcoplasmic protein represented the largest portion (about 50%) of the total nitrogen; however, it fell rapidly during the first 2 weeks after birth and then leveled off to 30% of total nitrogen at 4 weeks of age. At 8-12 days after birth, equal proportions of myofibrillar and sarcoplasmic proteins were observed. The relative amounts of extracellular protein (connective tissue) showed a steady but slight decrease with age and increasing body weight, the decrease being from 6.5% of total nitrogen at birth to 3.5% at 4 weeks of life.

III. The Effect of Growth on the Proportion of Muscle Protein to Muscle DNA

The decrease in the percentage of DNA in muscles with age and muscle weight is shown in Fig 1. The relative DNA content decreased from about 1.7% of dry muscles at birth to 0.24% at 2 months of age, with most of this change occurring during the first 2 weeks after birth. The absolute values for DNA content at the different muscle weights are indicated in Appendix III. The plot of N/DNA against dry muscle weight is shown in Fig. 10. A rapid increase in N/DNA was observed between birth and 4 weeks, indicating the protein was accumulating much faster than was DNA.

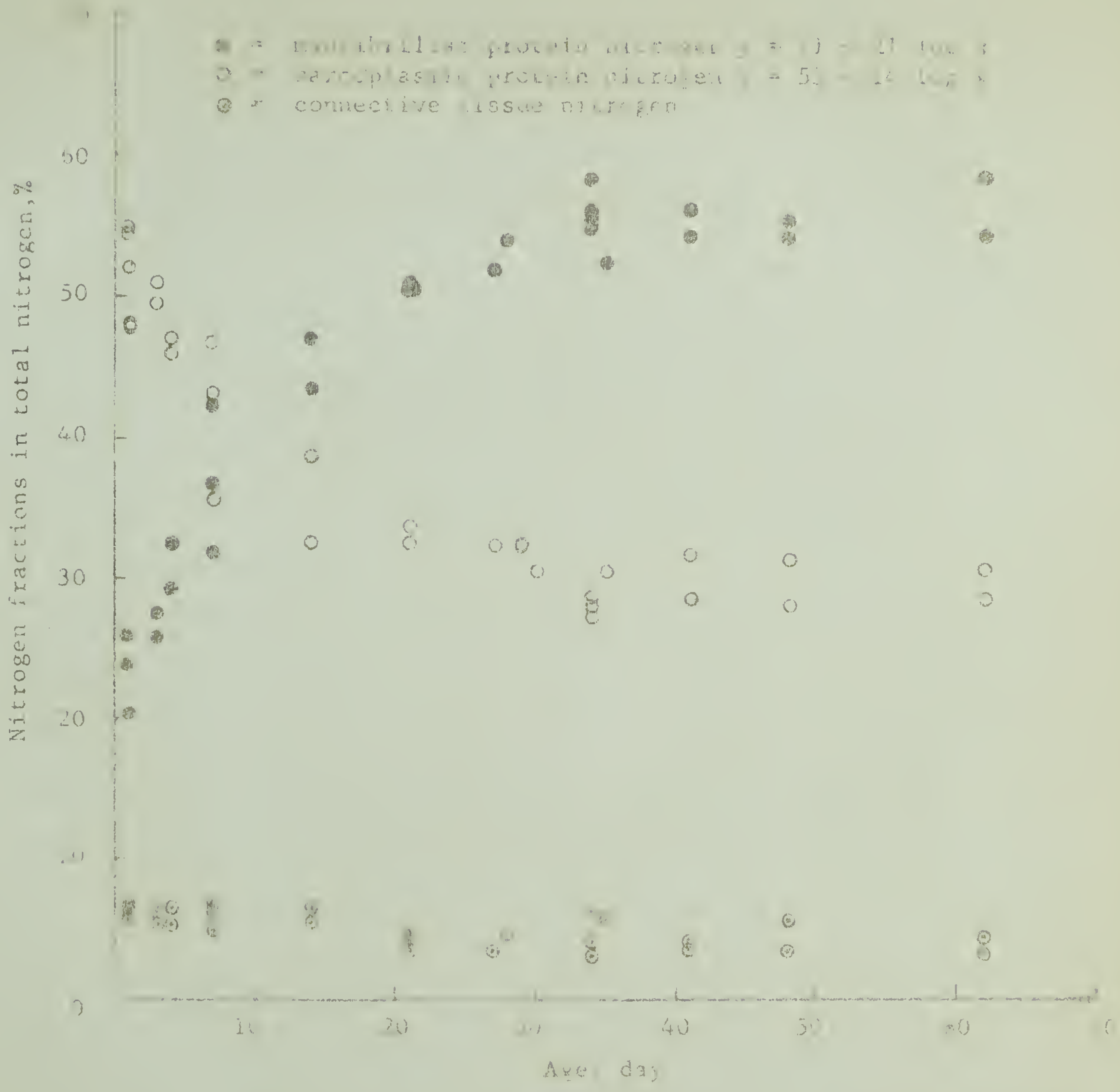

Fig. 2. Relative changes of muscle protein fractions in the right hind limb of growing rats. Experiment 1.

EXPERIMENT II

I. Growth Rate

A. Whole body

The growth curves of the rats in all treatments are shown in Fig. 3. The weights for individual rats are given in Appendix IV. The average initial weight of the animals was 53.7 gm. At the end of the experiment, the average weight for 8 controls was 265 gm, while that of the low plane rats was 124 gm, i.e., 47% of the controls. The low plane rats which were realimented with the undiluted stock ration beginning at 8 weeks of age, grew rapidly to reach 217 gm at 14 weeks, as compared to 265 gm and 124 gm for the controls and the remaining low plane rats respectively at 14 weeks of age.

B. Skeletal muscles

The growth rates of the left quadriceps muscles and of the right hind limb muscles are given in Appendix IV. The ratio of quadriceps muscles to body weight is indicated in Table 1 and Fig. 4. When comparisons were made on either a fresh or dry weight basis, the muscles increased in weight at a greater relative rate than did the body as a whole in all treatments. Restricted nutrient intake resulted in a higher apparent growth rate of muscles relative to body weight than the controls. There were, however, no differences in the relative growth rates of muscles between the treatments on the basis of fresh weight. The ratio of dry muscle to dry body weight appeared to reach a constant level in the control animals by the time their body weight had increased to about 40 gm, as indicated in Fig. 4 and Fig. 5. At 14 weeks of age, the absolute size of the muscles and the muscle weight to body weight values for the realimented rats approached those for control rats of equal weight.

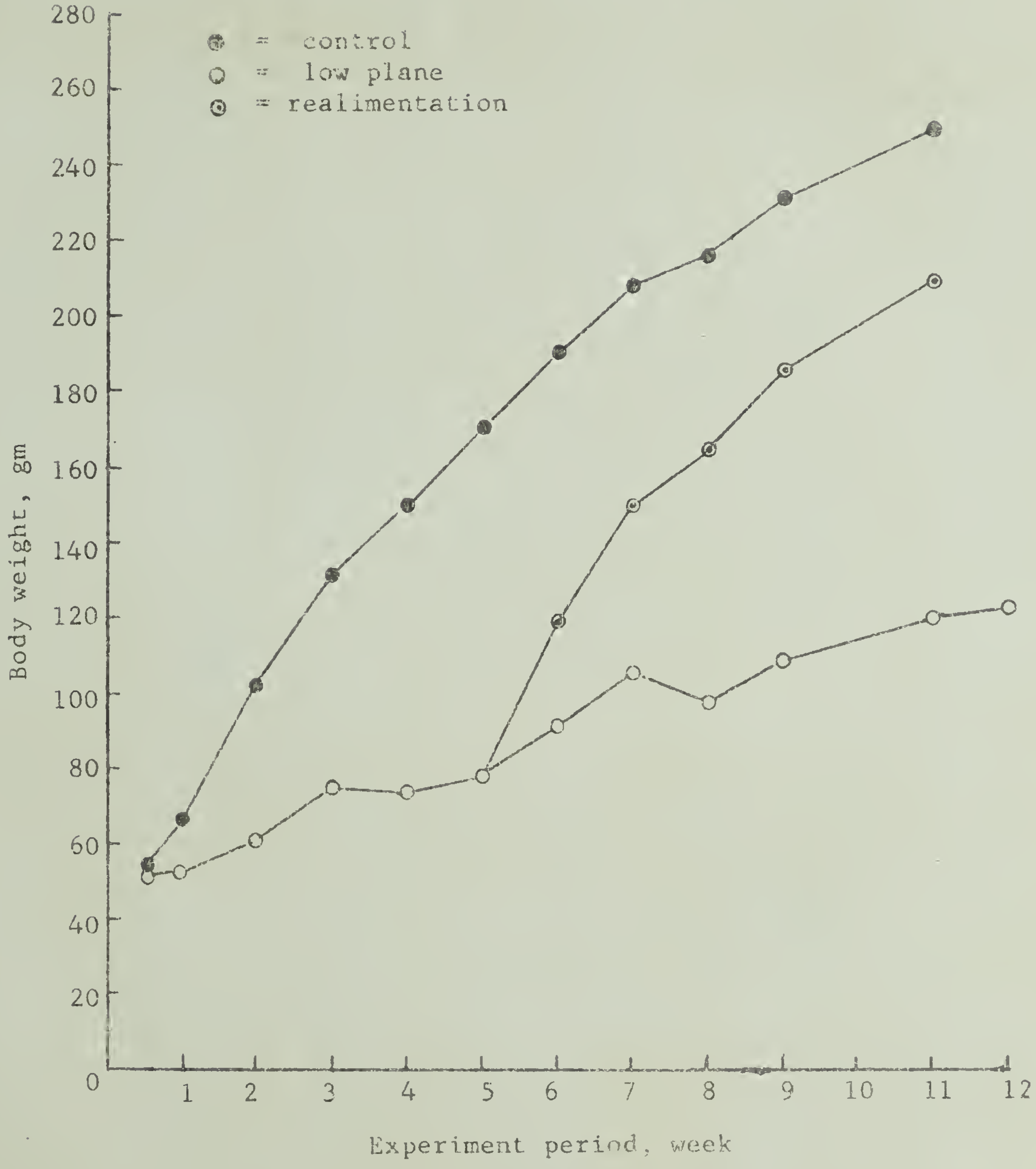

Fig. 3. Mean growth rates of rats as affected by the plane of nutrition.

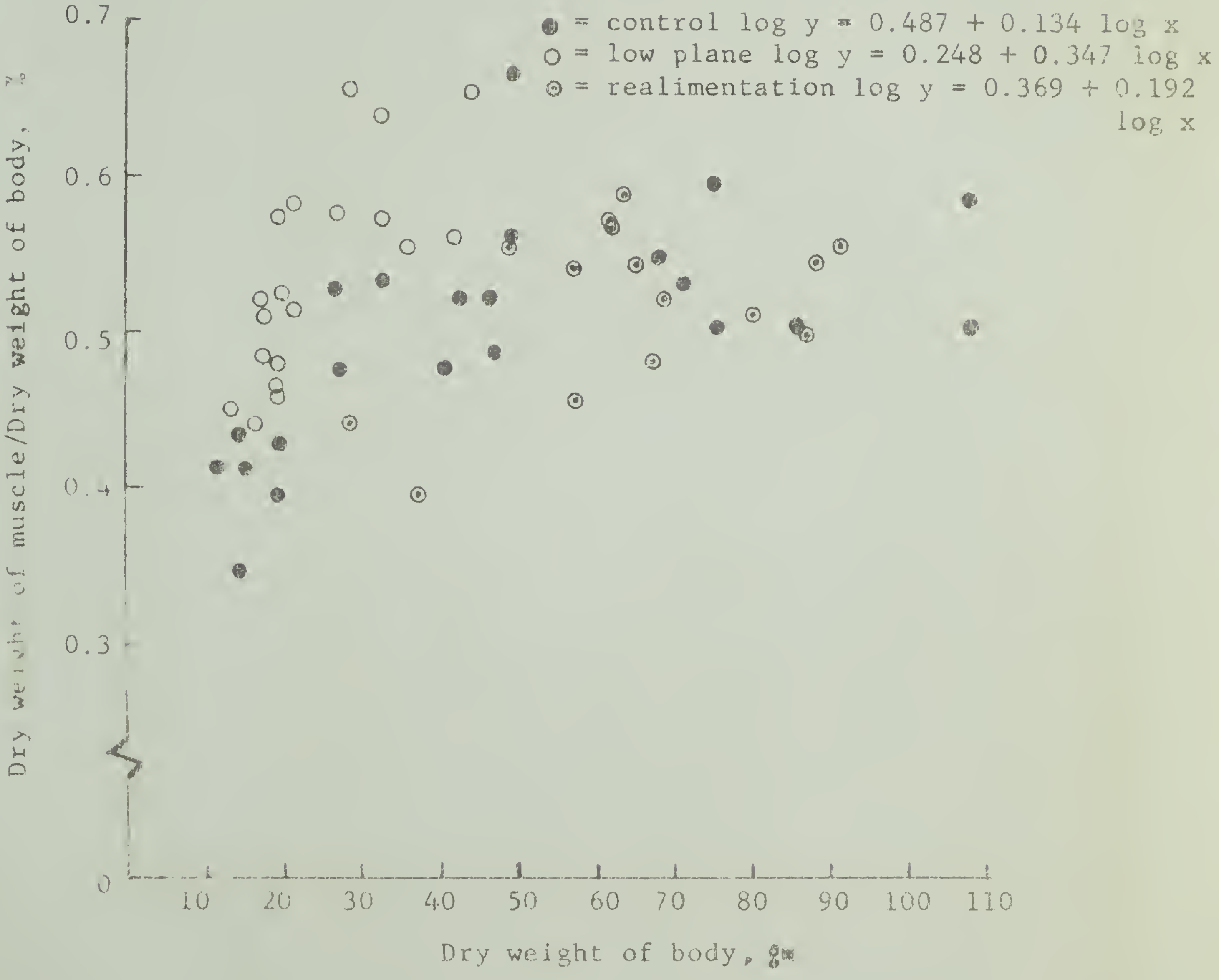

Fig. 4. The ratio of the weight of the left quadriceps muscles to dry body weight as a function of dry body weight.

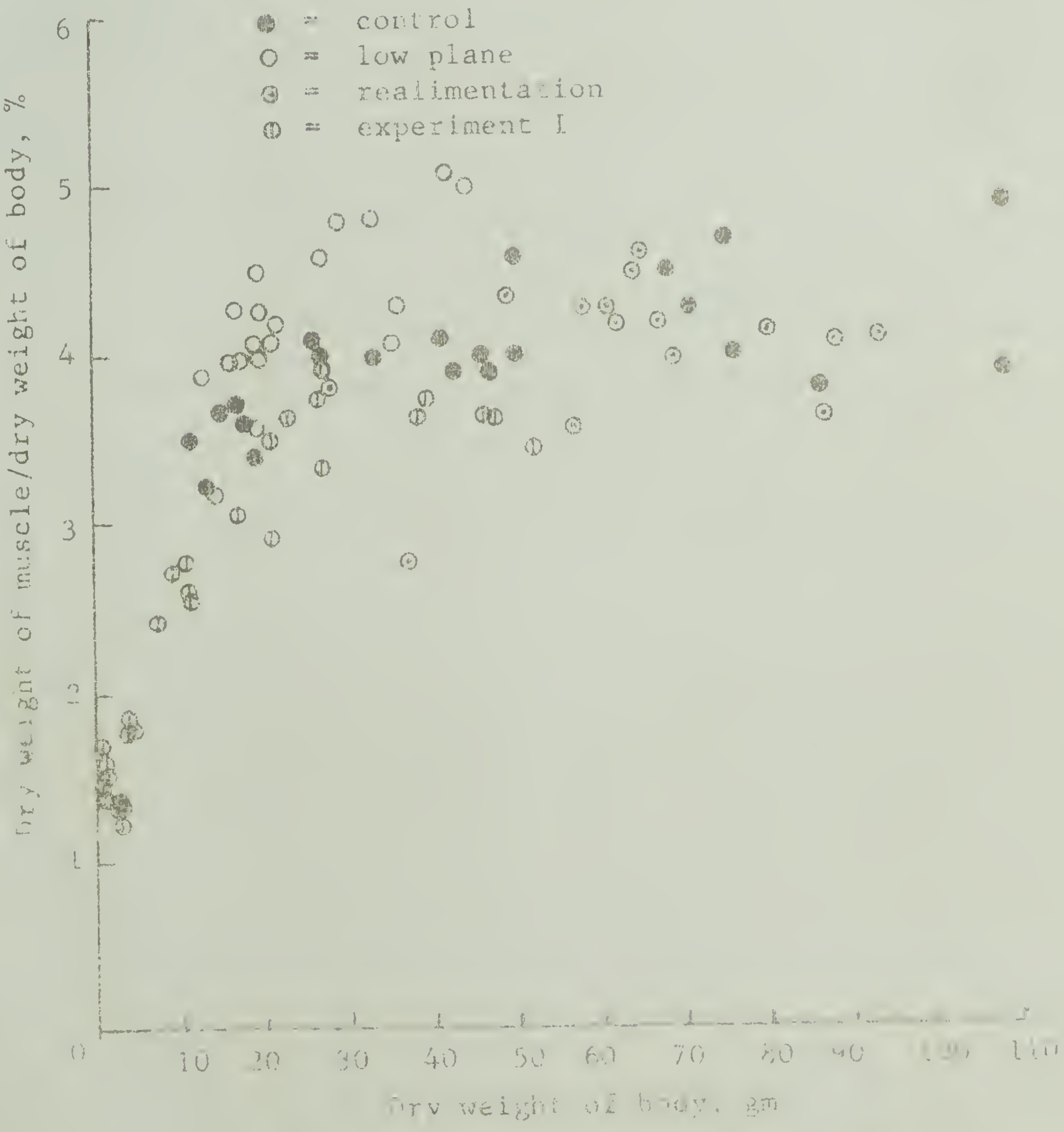

Fig. 5. The right hind limb muscle weight/body weight ratio as a function of dry body weight.

Table 1. Experiment II. Effect of plane of nutrition on the growth rate of the left quadriceps muscles

		Quadriceps muscles dry weight as % of dry body weight	Quadriceps muscles dry weight as % of initial control value
Initial control			
Dry body weight	13.9 gm	0.44	100
At dry body weight	34.6 gm		
Control		0.49	111
Low plane		0.61	139
Realimented		0.46	105
At dry body weight	69.2 gm		
Control		0.54	123
Realimented		0.53	120

II. Effect of Growth on the Composition Changes

A. Whole body

Table 2 and Appendix IV illustrate the effects of the different treatments on water and nitrogen content. The relative water content of the whole body decreased in all treatments as compared with that of the initial control. Restricted feeding resulted in a higher water content relative to control animals of the same age. In all treatments, however, changes in body water showed a similar pattern relative to dry body weight. The changes in the percent total nitrogen in the whole dry body with age and between treatments were not signficant, although the low plane rats tended to have the highest relative nitrogen contents.

There was a steady increase in the ratio of N/H_2O as dry body weight increased for the animals in all treatments although this occurred at a progressively decreasing rate (Fig. 6). Restricted feeding resulted in a lower ratio on the basis of age, but resulted in a slightly higher value on a weight basis. In general, however, the N/H_2O ratio for the restricted rats followed a similar pattern relative to dry body weight

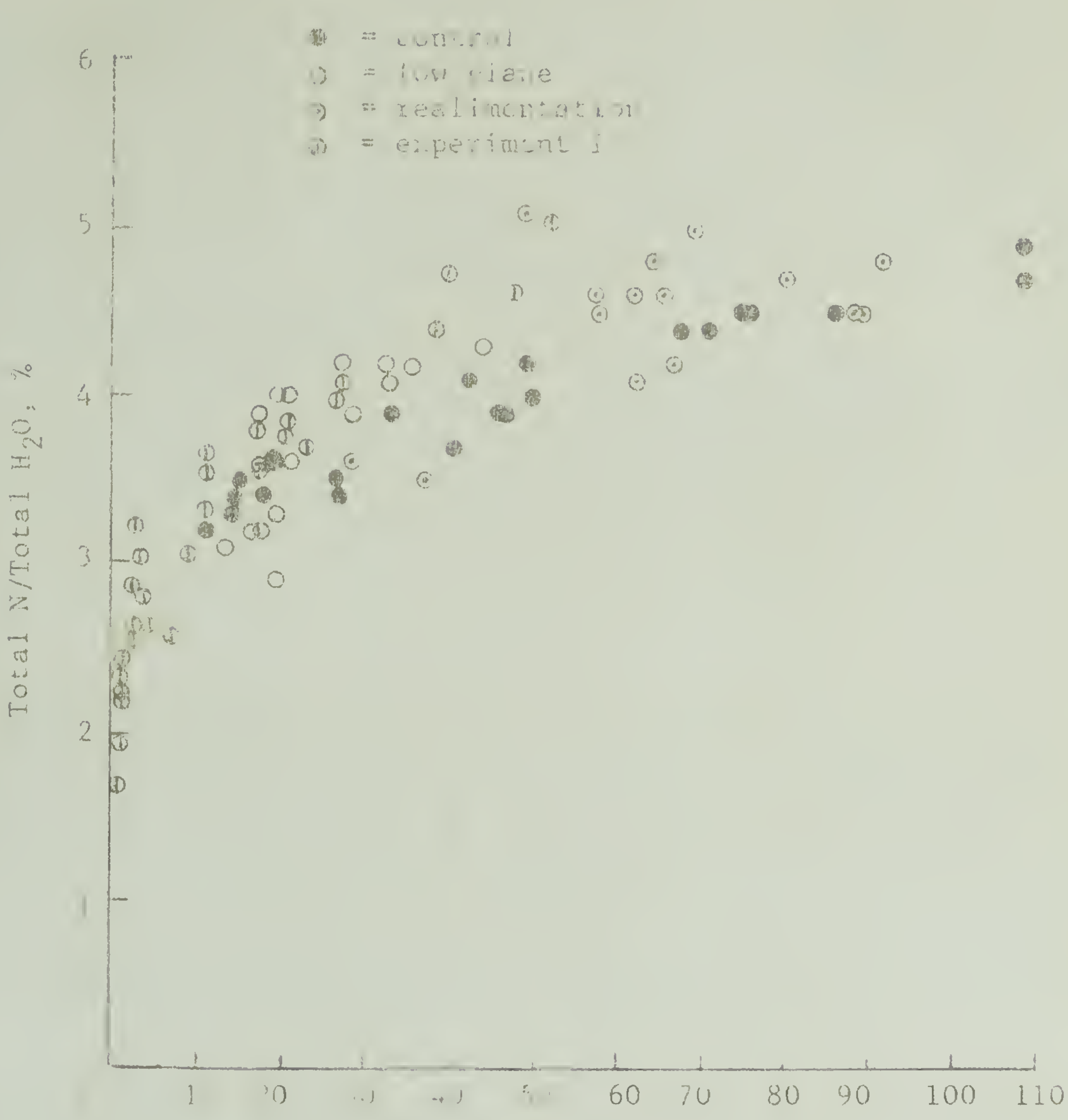

Fig. 6. The whole body N/H_2O ratio as a function of dry body weight. Experiments I and II.

Table 2. Experiment II. Effect of plane of nutrition on the water and nitrogen content of the whole rat

	Initial control	Control	Low-plane	Realimented low-plane
Age (days)	26	97	102	100
Dry body weight (gm)				
Average	13.9	84.5	34.6	69.1
Range				
Water (% of whole body)				
Average	74.03	68.11	72.07	68.16
Range	73.1-75.5	66.1-69.8	71.2-73.5	66.0-70.8
Nitrogen (% of dry body weight)				
Average	9.73	9.76	10.74	9.87
Range	9.6-10.0	8.8-11.0	10.2-11.1	9.0-11.4

to that of the controls.

B. Skeletal muscles

As Appendix IV indicates the decrease in the proportion of water in the quadriceps muscles with age was not as great as that which occurred in the whole body. Similarly, in the low plane rats, restricted feeding had less effect on muscle water than on total body water.

The rate of increase in N/H_2O ratio in quadriceps muscles as the muscles increased in weight was not as great as that of the whole body. There was almost no difference between the treatments on an age basis (Fig. 7). The proportions of water and nitrogen, and the N/H_2O ratio versus dry muscle weight relationship were similar in the combined whole hind limb muscles to the values obtained for the quadriceps muscles. The analytical results are indicated in Appendix V.

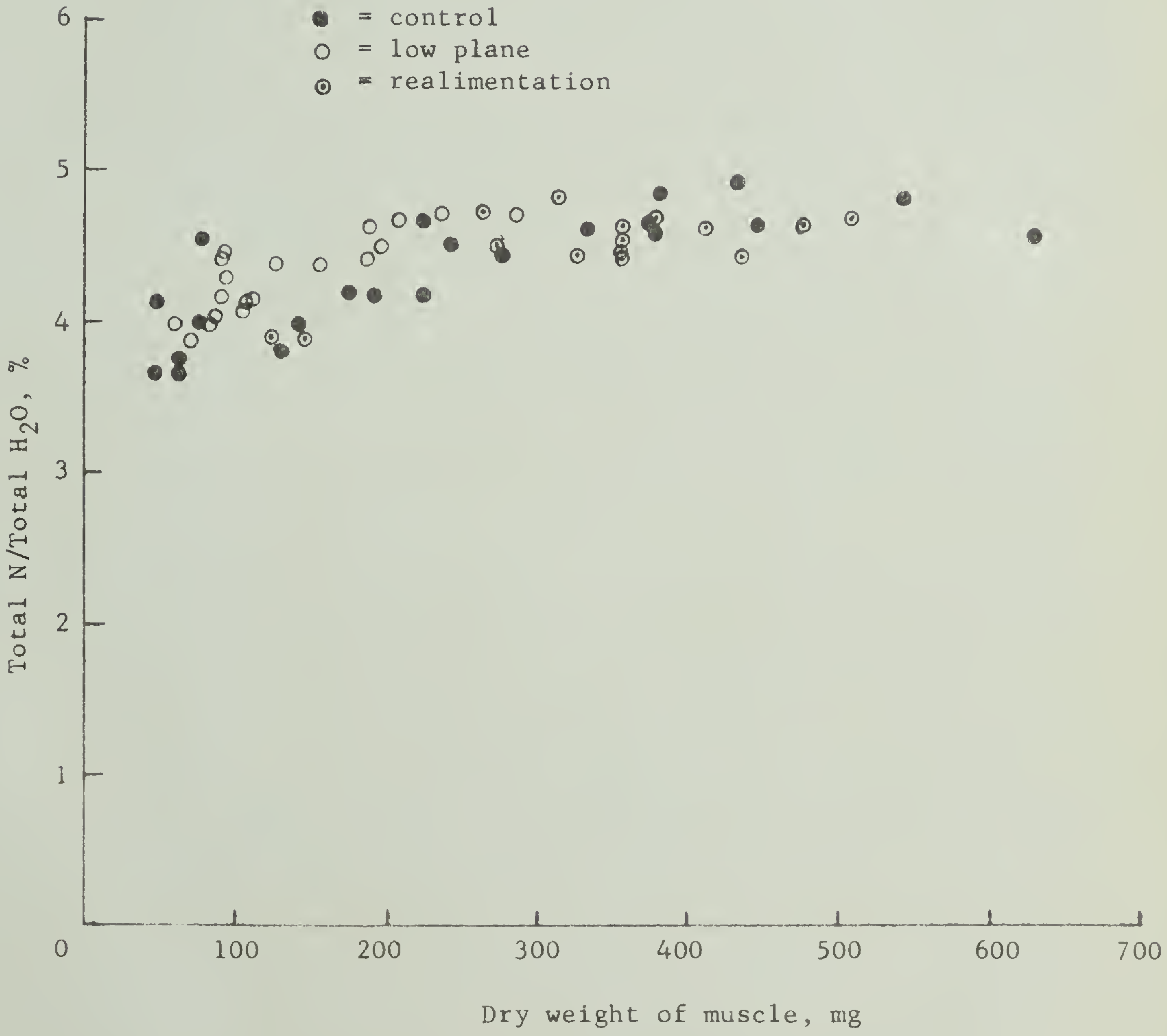

Fig. 7. The N/H_2O ratio of the quadriceps muscles as a function of the dry weight of the muscle.

III. Muscle DNA changes in Relation to Muscle Growth

The DNA content of the muscles of the right hind limb was estimated in 44 rats. The values obtained from ultra-violet absorption were slightly lower than those from phosphorus determination; this was probably a function of the methods used as the former directly measures the DNA or DNAP content while the latter indirectly measures DNAP. The results obtained using this latter, indirect method were used in plotting all changes in muscle DNA in relation to muscle growth. As Fig. 8 indicates, the proportion of DNA gradually decreased as the muscles increased in weight. Table 3 shows the relative content of DNA expressed on a dry weight basis. Restricted feeding resulted in a higher value than for control rats on equal age, but this treatment did not change the proportion of DNA when restricted and control rats were compared on an equal weight basis. The relative content of DNA in the muscles of realimented rats decreased to approach the values for the controls as the body weights of the realimented rats approached those of the controls.

The absolute content of the DNA in muscle as a function of dry muscle weight is shown in Fig. 9. Although restricted feeding resulted in a higher relative DNA content, it gave a lower absolute value of DNA due to less muscle gain.

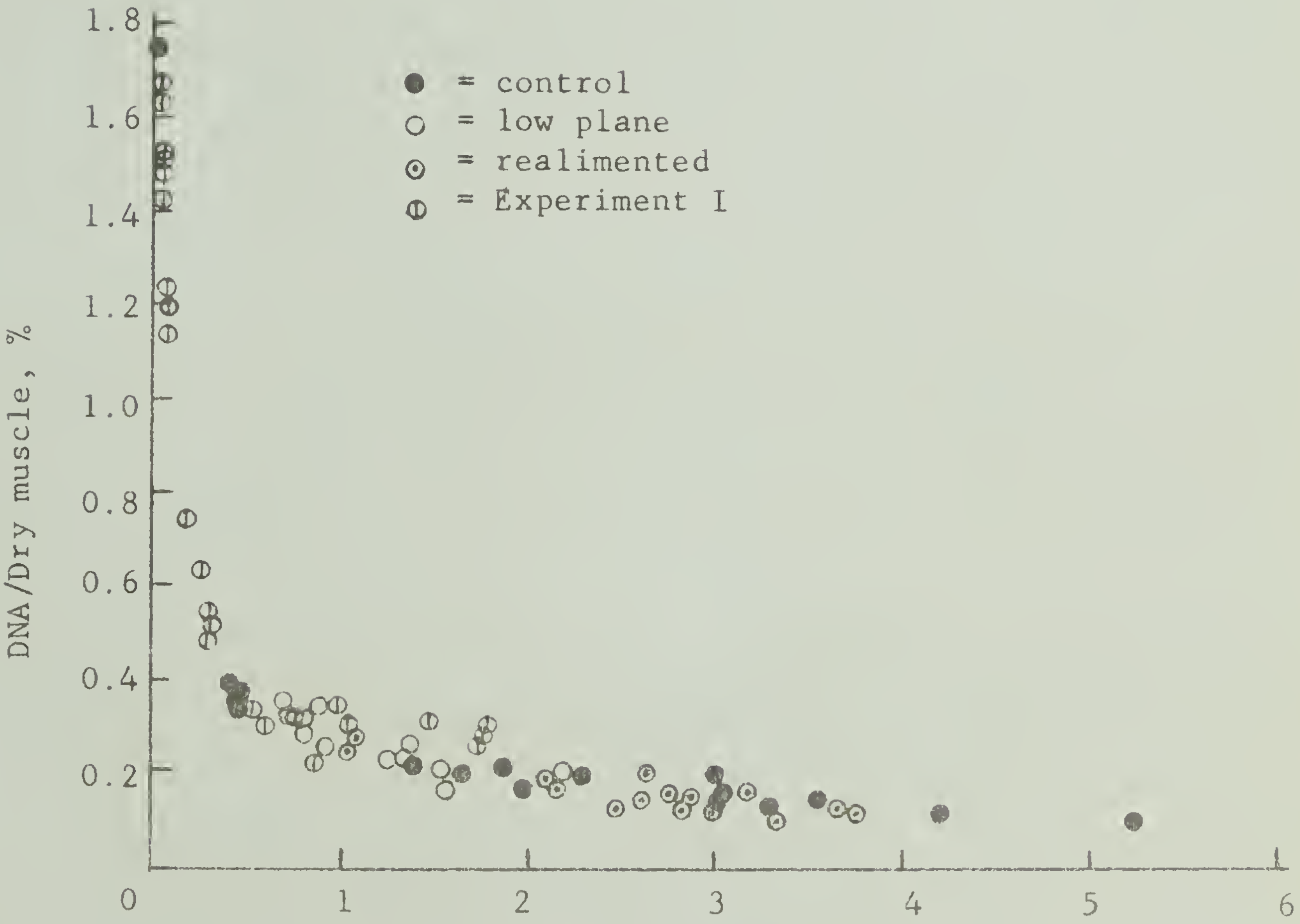

Fig. 8. The percentage of DNA in dry muscle as a function of dry muscle weight.

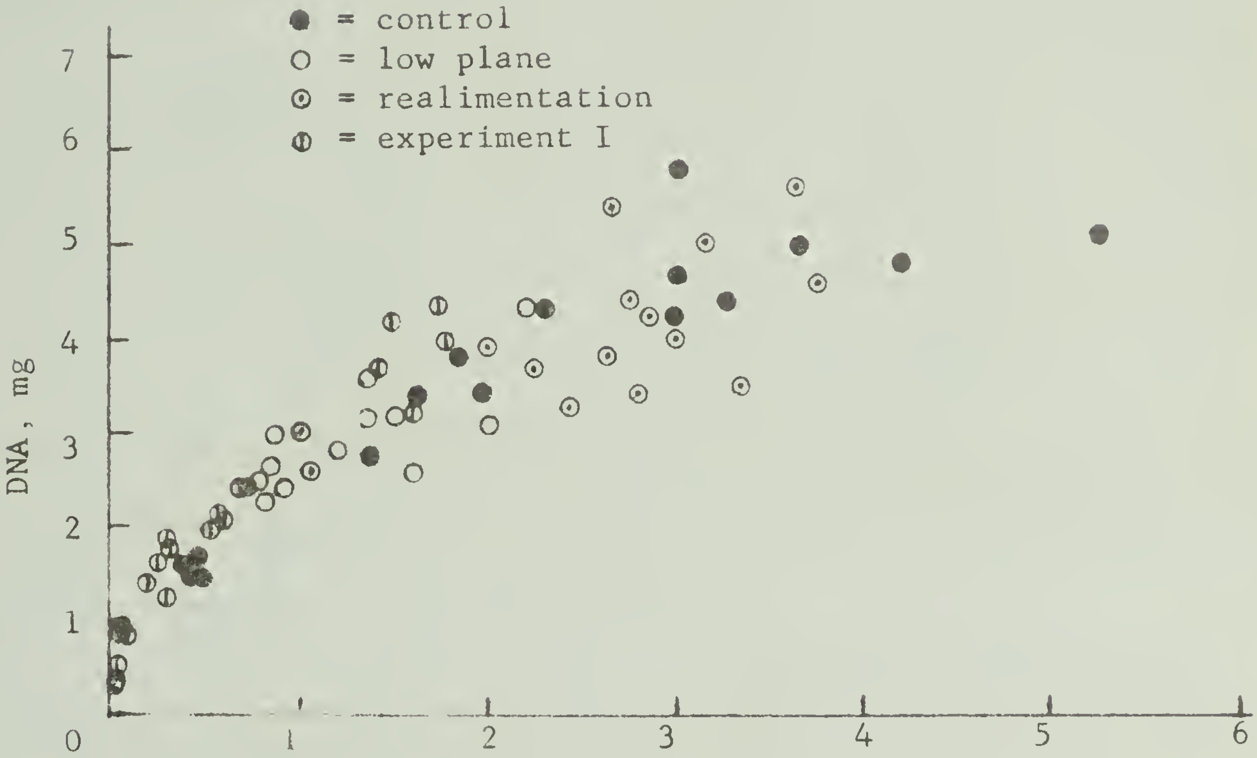

Fig. 9 Total DNA in dry muscle as a function of dry muscle weight.

Table 3. Experiment II. Effect of plane of nutrition on the DNA content of the right hind limb muscles of the rats

	Initial control	Control	Low-plane	Realimented low-plane
Age (days)	26	97	102	100
Dry body weight (gm)	13.9	84.5	34.6	69.1
DNA content (% of dry muscle weight)				
Average	0.36	0.14	0.21	0.15
Range	0.34-0.39	0.15-0.19	0.15-0.26	0.10-0.20

The N/DNA ratio increased, generally, in a reasonably linear fashion as muscles increased in weight except during the initial period of growth in which N/DNA ratio showed a seemingly greater rate of increase with the increase of muscle gain (Fig. 10). Restricted feeding retarded the rate of change in N/DNA ratio with age, but did not affect the change in this ratio, relative to dry muscle weight.

The myofibrillar protein content of the right hind limb muscles of the 44 rats was also determined. The analytical results are presented in Appendix V. The myofibrillar protein N/DNA ratio for the right hind limb muscles plotted as a function of dry muscle weight is shown in Fig. 11. The relationship is similar to that for the total protein nitrogen, and as in the latter case, the plane of nutrition did not appear to alter the rate of change of myofibrillar protein N/DNA with respect to dry muscle weight.

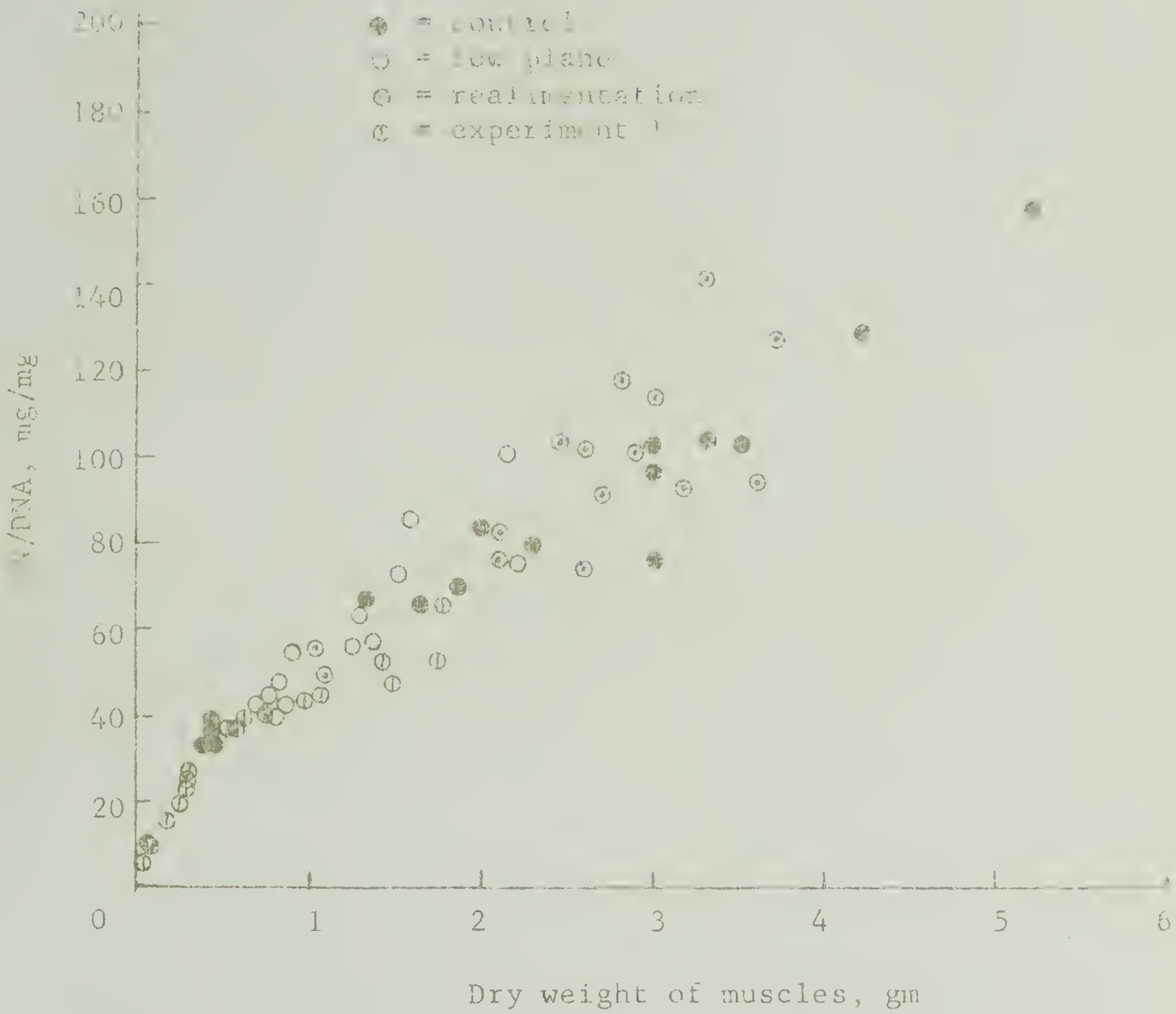

Fig. 10. Total nitrogen relative to total DNA as a function of dry weight in the right hind limb muscles. Experiments I and II.

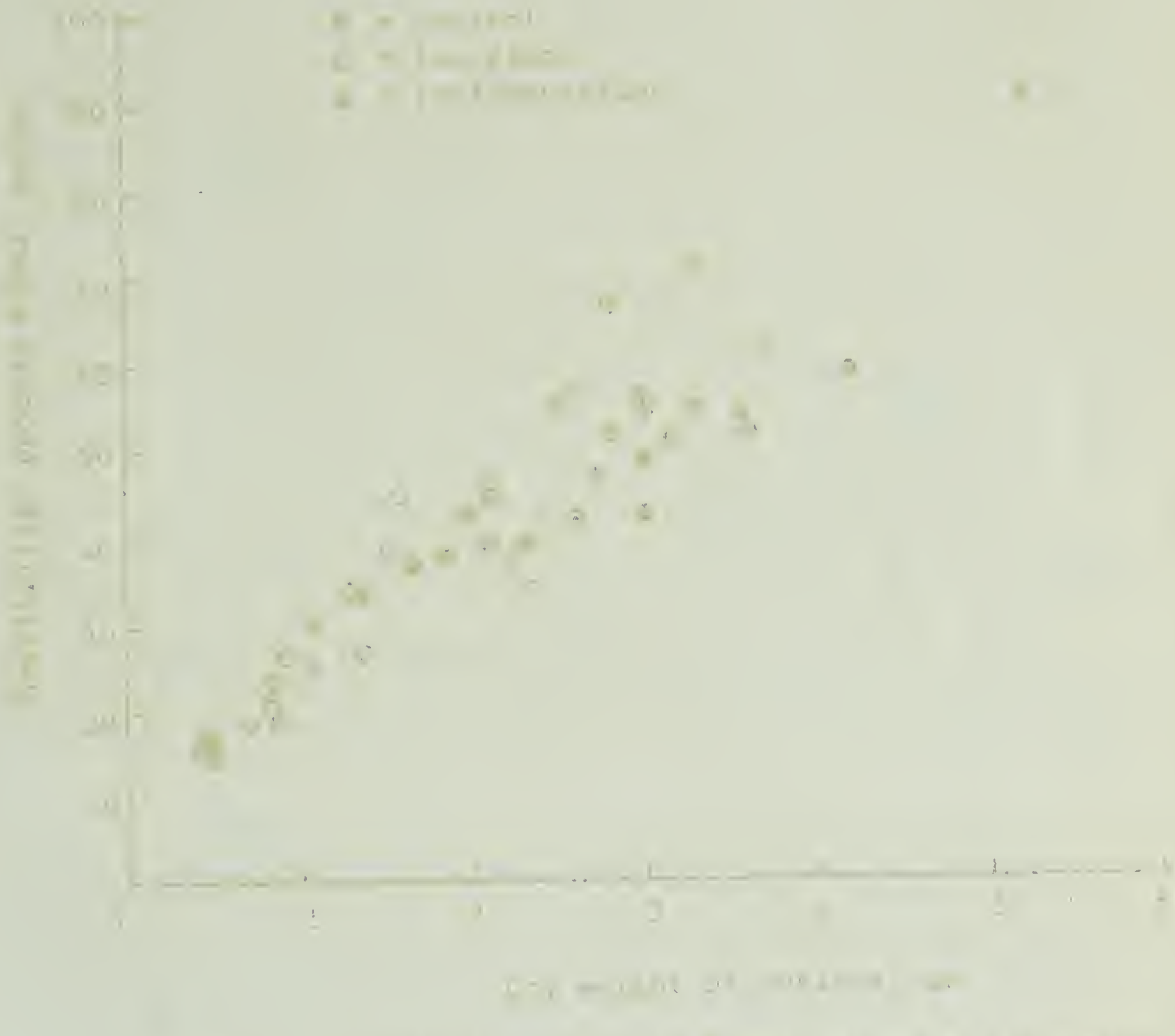

Fig. 11. [illegible] as a function [illegible] weight of the [illegible] time.

DISCUSSION

I. Muscle Growth

The increase in the ratio of muscle weight to body weight which was observed in all of the groups is undoubtedly related to the physiological maturation of the rat. The fact that the dry muscle/dry body weight ratio increased more rapidly in the low plane rats than in the control or realimented low plane rats would seem to be due to a lower rate of fattening in the former, so that muscle in the former group comprised a greater proportion of the total body mass. In this respect, McMeekan (1940) demonstrated a higher proportion of muscle and a lower proportion of body fat in low plane pigs. Realimenting allowed accelerated muscle growth and fattening in previously restricted rats with the result that the dry muscle weight/dry body weight ratio of these animals approached the values for the controls of equal body weight.

It seems possible that had muscle and whole carcass been fat extracted, the resulting differences in fat-free dry muscle weight/fat-free dry body weight ratio between the low plane rats and the other groups would have been minimal.

II. Changes in Water and Nitrogen in Whole Body and Muscle

The relationship between total nitrogen and total body water has been suggested as an index of physiological age (Keys and Brozek, 1953; McCance and Widdowson, 1956; Bailey et al, 1960). In the present experiments, total N/H_2O increased rapidly in the immediate post-natal period, but the rate of increase diminished. Thus, at a dry body weight of about 60 gm, the ratio

became almost constant, and with further growth, nitrogen and water increased at about the same rate. The N/H_2O changes in the quadriceps muscles were similar to, but of smaller magnitude than those in the whole body.

There have been indications that physiological aging could be postponed by restricted nutrition (McCay, Crowell and Maynard, 1935; Lansing, 1951; Bailey et al, 1960).

In the present experiments, the low plane of nutrition slowed the rate of change in N/H_2O relative to chronological time. Thus, when compared at constant age, the low plane rats were physiologically less mature than the controls. The slightly increased rate of change of N/H_2O as a function both of dry muscle mass and of dry whole body weight in the low plane group was probably due to a lower muscle and carcass fat content since N/H_2O curves for the control and realimented animals were similar.

In the present experiments the restricted nutrient intake reduced the overall growth rate to about 50% of that of the control rats--a degree of restriction which would not be expected to cause the edematous conditions which could occur in cases of severe protein malnutrition, and which would produce abnormally low nitrogen/water ratios.

III. The Protein Distribution in Muscles

The results indicated that development and growth of skeletal muscles were associated with a differential distribution of protein components. The rapid increase of myofibrillar protein during the first 4 weeks after birth would correlate with the developing functional activity of muscles, i.e., in the development of contractile

protein for physical movement. The post natal changes in the muscle protein fractions varies with species as demonstrated in the literature. In the case of the rats in the present experiment, the protein components of muscle in control animals reached a constant level after about 1 month of age, while in cattle this stage of development is reached at about 5 months of age (Lawrie, 1961b).

The much lower proportion of myofibrillar protein found at birth in rats than in calves, reflects the fact that new-born calves are more physiologically mature than new-born rats. The gradual decrease in the relative proportion of connective tissue protein from birth to maturity was in agreement with the results of other investigations on various species (Robinson, 1952b;Dickerson, 1960; Dickerson and Widdowson, 1960; Lawrie, 1961b).

The chemical determination of protein fractions is in coincidence with histological observations. It has been demonstrated that the size of intracellular muscle mass increased at a faster rate than muscle connective tissue during growth (Chiakulas and Pauly, 1965).

The protein distribution in muscle could be affected by the plane of nutrition if the comparisons are made on an age basis. Restricted feeding, as observed in experiment II, resulted in a slightly lower content of myofibrillar protein relative to control rats of equal age; this would be accompanied by correspondingly higher levels of connective tissue protein.

IV. Changes in the Content of Muscle DNA

It has been demonstrated that the relative DNA content of muscle decreased rapidly after birth in the rats (Herrmann and Nicholas, 1949) and in the chicks (Herrmann, 1952 and Robinson, 1952b). The present

experiment has shown similar results except there are some deviations in the extent of the changes in the relative DNA content. This is in coincidence with histological observations--the relatively larger nuclei in young muscle cells resulting in a higher nucleo-cytoplasmic ratio (Dickerson and Widdowson, 1960). The results of the present experiment are also coincident with the findings of Enesco (1960, 1961) and of Enesco and Leblond (1962)--the weight of muscles increased more rapidly than the DNA content in the muscles.

Quite variable contents of DNA in the skeletal muscle have been reported recently in the literature. Without specifying the experimental conditions it seems unreasonable to compare the values. The DNA content in hind limb muscles of rats at 4 weeks of age was 0.07% of the fresh weight as found in the present experiment. This value was similar to that of Mendes and Waterlow (1958). Restricted feeding resulted in a higher content of DNA on an age basis in experiment II. However, the low plane rats generally followed the same pattern as controls when DNA levels were expressed on a weight basis, indicating that these animals were physiologically less mature than controls of the same age. Various experiments have demonstrated that the animals suffering from severe malnutrition had a higher muscle DNA content than did normal control adults, and approximately the same as that of initial controls (Mendes and Waterlow, 1958; Montgomery et al, 1964). Realimenting the restricted animals resulted in accelerated growth and concomitantly restored the relative DNA content to that of controls having the same age and weight. This was in agreement with the results of Montgomery, et al (1964).

V. The Ratio of Nitrogen to DNA and of Myofibrillar Protein Nitrogen to DNA

The N/DNA ratio was taken as an index of the accumulation of nitrogenous components in a cell unit. The most rapid increase in this ratio was found during the first 2 weeks of growth, as indicated in experiment I. Histologically, it has been demonstrated that the ratio of cytoplasm-to-nucleus in adult muscle is much greater than that in young animals (Dickerson and Widdowson, 1960; Enesco and Leblond, 1962). Restricted feeding resulted in lower N/DNA values on an age basis as observed in experiment II. On a weight basis, however, the N/DNA ratio in low plane animals followed a similar pattern to that of the controls, indicating once again that the rate of physiological maturation was retarded by restricted feeding. Realimenting the low plane rats and subsequent increase in weight resulted in an increase in the rate of change in the N/DNA ratio. It has also been shown that the N/DNAP ratio (Mendes and Waterlow, 1958) and the protein N/DNAP ratio (Montgomery, Dickerson and McCance, 1964) remained static when animals were subjected to severe undernutrition, and that rehabilitation restored those ratios. Histological studies indicated that the muscle cell is multinucleated and there is increase in number of nuclei during growth (Enesco, 1960, 1961). Moreover there is still an unsolved problem as to the extent, if any, of hyperplasia occurring (Chiakulas and Pauly, 1965). In consideration of these facts, chemical measurements in terms of N/DNA and/or Cell Mass/DNA would be employed was indices of Nitrogen and/or cell mass per nucleo-cytoplasmic unit respectively without involving the term hyperplasia.

SUMMARY AND CONCLUSIONS

1. The skeletal muscles (quadriceps muscles and hind limb muscles) had a higher relative growth rate than the body as a whole. Restricted feeding resulted in a higher muscle/body weight ratio due apparently to the decreased content of body fat in the low-plane rats. Realimented low-plane rats at 217 gm of body weight had a similar muscle/body weight ratio to controls of the same weight.

2. As in the case of the whole body there was also an increase of nitrogen and decrease of water in the muscles during the early period of growth.

3. Differential growth occurred in the different protein components of muscle during the first month after birth. Myofibrillar protein increased at a faster rate than sarcoplasmic protein, while the connective tissue exhibited the slowest rate of increase.

4. There was a relative decrease in the proportion of DNA in the muscles during growth, and the most rapid decrease was found during the first two weeks of age.

5. The accumulation of total nitrogen and myofibrillar protein in a cell unit occurred most rapidly during the early period of life.

6. Restricted feeding did not appear to alter the rate of physiological maturation with respect to weight but did retard physiological aging relative to chronological time. Thus, the low-plane rats were in a more immature stage in terms of the ratios of N/H_2O, N/DNA and myofibrillar protein N/DNA when compared to controls of similar chronological age.

7. Realimentation of the restricted rats resulted in an acceleration of muscle and whole body growth, so that the chemical composition of these animals at a body weight of 217 gm was similar

to that of controls of the same weight. It appeared then that by the criteria studied, the period of dietary restriction decreased the rate of physiological aging, but did not lead to irreversible changes in the nature of muscle growth.

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APPENDIX I

BASAL RAT DIET, DEPARTMENT OF ANIMAL SCIENCE, UNIVERSITY OF ALBERTA

Ingredients	%	Protein %	Lysine %	Methionine %	Cystine %	Tryptophan %	TDN %	Dig. Energy Kcal./kg.	Ca. %	P. %	Mn. mg./kg.	Vit. A I.U./kg.
Wheat	17.0	2.3	.06	.03	.03	.03	13.6	598	.01	.07	7.5	
Barley	23.1	2.7	.11	.04	.04	.04	16.1	708	.01	.09	4.1	
Oats	25.0	3.0	.09	.05	.05	.04	16.5	726	.02	.08	11.0	
Soybean oil meal	13.0	5.7	.33	.10	.08	.07	10.0	440	.03	.08	3.6	
Fish meal	4.0	2.9	.25	.08	.04	.04	2.8	123	.12	.08	0.4	
Linseed oil meal	5.0	1.6	.06	.04	.03	.03	3.5	154	.02	.04	1.9	
Meat meal	3.0	1.7	.10	.02	.02	.02	1.9	84	.24	.12	0.3	
Wheat bran	5.0	0.8	.03	.01	.01	.01	2.5	110	-	.06	5.5	
Alfalfa meal	3.0	0.5	.03	.01	.01	.01	.7	3.	.04	.01	1.0	
Iodized salt	0.5											
Ground limestone	0.8								.31			
Bonemeal	0.25								.06	.03		
Vit. B-complex mix	0.1											
Vitamin A	+											1100
Vitamin B_{12}	0.1											
Vitamin D_2	+											
Feeding oil (1000 A, 150 D)	0.15											1500
	100.00	21.2	1.06	.38	.32	.29	68	2974	.86	.66	35.3	2600
Per lb.								1351			16	1180

Ingredients	Vit. D_2 I.U./kg.	Thiamine mg./kg.	Riboflavin mg./kg.	Niacin mg./kg.	Pantothenic acid mg./kg.	Pyridoxine mg./kg.	Choline mg./kg.	Vit. B_{12} g./kg.	Fiber %	Fat %	Energy Kcal./kg.	Prod. Energy Kcal./kg.
Wheat		1.20	.15	9.0	1.9	.5	140		0.5	.3	561	381
Barley		1.40	.40	13.0	1.3	.9	240		1.6	.4	650	406
Oats		1.54	.26	4.1	2.7	.8	240		3.0	1.0	655	418
Soybean oil meal		.85	.37	3.5	1.7	-	340		0.8	.1	292	163
Fish meal		.05	.35	3.5	.4	.6	160	8	-	.3	119	82
Linseed oil meal		.30	.18	2.0	.7	-	110		0.4	.2	76	56
Meat meal		.01	.11	1.6	.1	-	60	2	0.1	.2	72	50
Wheat bran		.40	.11	1.4	.3	-	50		0.5	.2	57	53
Alfalfal meal		.09	.33	.8	.8	-	25		0.9	.1	30	16
Vit. B-complex mix			4.40	19.8	8.8		22					
Vitamin B_{12}								20				
Vitamin D_2	220											
Feeding oil (1000A, 150D)	220											
	440	5.84	6.66	58.7	18.7	2.8+	1387	30	7.8	2.8	2512	1625
Per lb.	200	2.65	3.03	26.7	8.5	1.3+	630	14			1142	739

APPENDIX II

I. The Fractionation of Muscle Protein

A. The direct determination of myofibrillar protein (Robinson, 1952a)

1. A sample of fresh muscle was minced with scissors in cooled extractant solution (1.25 M KCl, 0.066 M K_2HPO_4, pH 8.5, ionic strength, 1.45).
2. The sample was homogenized in an ice bath, and centrifuged for 1 hr at 693 g. This extraction process was repeated twice.
3. The three combined salt extracts were diluted with 0.066 M NaH_2PO_4 and water to a final KCl concentration of 0.5 M and ionic strength 0.58 at pH 7.1.
4. The combined extracts were further diluted with 19% ethanol (V/V) to ionic strength 0.12 and a final ethanol concentration of 15% (V/V). The temperature was kept at 0^oC through these additions.
5. The precipitated protein was centrifuged and washed twice with 5% trichloroacetic acid (W/V).
6. The nitrogen content of the precipitate was then determined.
7. The residue after extraction with strong salt solution was twice extracted with 0.1 N NaOH by homogenization and then allowed to stand overnight in 0.1 NaOH at 0^oC.
8. The nitrogen content of this combined extract was then determined.
9. The nitrogen from steps 6 and 8 equalled the myofibrillar protein nitrogen.

B. Modification of Robinson's method (Steward, 1955).

1. The minced tissue was homogenized in ice cold extraction solution (0.1 M KCl, 0.0277 M K_2HPO_4, 0.0163 N NaH_2PO_4, pH 7.1, ionic strength 0.2).
2. The homogenate was separated by centrifugation. The residue was homogenized and centrifuged twice more as described in step 1.
3. The three combined supernatant solutions were made up to a volume of 25 ml, then diluted with an equal volume of 30% (V/V) ethanol added from a fine capillary at 0°C.
4. A small precipitate of actomyosin was removed by centrifugation.
5. The determination of nitrogen in the supernatant gave the sarcoplasmic protein nitrogen.
6. The residue after extraction of sarcoplasmic protein was homogenized with 0.1 N NaOH twice and was allowed to stand overnight in 0.1 N NaOH. The residue was washed with 0.1 N NaOH and allowed to stand one more night in 0.1 N NaOH at 2°C.
7. The nitrogen in the residue was then considered to be connective tissue protein nitrogen.
8. The myofibrillar protein was then calculated as follows: Total nitrogen - (Sarcoplasmic nitrogen + connective tissue nitrogen) = myofibrillar protein nitrogen.

II. The Determination of DNA

A. Removal of acid-soluble and lipid phosphorus.

1. Fresh muscle (0.2-1.0 gm) was minced in 20 volumes of 7%

trichloroacetic acid.

2. The suspension was homogenized for 20 min.
3. The residue after filtration was washed with 1% trichloracetic acid until free of inorganic phosphate.
4. The residue was then washed until the filtrate was only weakly acid toward litmus paper.
5. The residue was then washed in alcohol and ether.
6. The residue was suspended in 30 to 40 volumes of a mixture of alcohol and ether (75:25) and boiled for a few minutes.
7. The residue was then refluxed for 30 min. in 30 to 40 volumes of a boiling mixture of equal volumes of methanol and chloroform.
8. The residue was then washed in ether and then dried in an evacuated dessicator.

B. Treatment with alkali and determination of phosphorus

1. The dry residue was incubated in 1 N KOH for 18 hr. at 37°C.
2. Aliquots were taken for total phosphorus determination.
3. Another aliquot was precipitated with 0.2 volume of 6 N HCl and 1 volume of 5% trichloracetic acid. The phosphorus content of the filtrate was then determined.
4. The DNA-phosphorus was then calculated as follows:

   Total P - filtrate P = DNAP.

C. Ultraviolet determination of DNA

1. The precipitate from the acid treatment (B, 3) was treated with 5% trichloroacetic acid at $90^{o}C$ for 15 min.
2. The DNA in the supernatant solution was measured in a Beckman DU spectrophotometer at 268.5 mu.

APPENDIX III

Body weight, muscle weight and chemical compositions of muscles. Experiment I.

		Whole body			Muscle of		
Rat no.	Age day	Fresh weight gm	H_2O %	Total N mg	Fresh weight mg	H_2O %	Total N mg
1	1	6.2	81.3	112	125	86.5	1.8
2	1	6.4	81.2	113	130	85.5	1.9
3	1	6.5	81.3	128	132	86.9	1.8
4	1	6.9	82.4	111	145	88.3	1.8
5	1	5.4	83.6	90	118	87.1	1.7
6	1	5.5	81.4	104	120	86.4	1.9
7	3	10.9	78.7	223	181	83.0	3.4
8	3	12.1	78.5	241	200	83.9	3.7
9	4	11.0	77.6	276	195	83.2	3.7
10	4	10.5	78.5	237	176	82.9	3.6
11	7	16.2	76.7	377	351	81.1	8.0
12	7	17.1	76.8	369	372	80.1	8.0
13	7	17.9	77.2	364	381	80.8	7.8
14	14	32.0	76.6	629	875	79.3	20.6
15	14	38.3	76.3	891	1200	79.4	30.6
16	21	44.7	75.5	1098	1415	78.5	38.9
17	21	45.5	74.6	1190	1402	78.7	38.3
18	21	45.3	74.3	1234	1410	78.9	39.3
19	27	74.9	72.7	2045	2819	78.6	81.4
20	28	66.4	73.8	1861	2644	79.7	72.0
21	34	77.3	73.1	2161	3350	78.3	98.1
22	34	99.3	72.6	2938	4902	77.2	144.1
23	34	88.1	73.2	2348	3754	77.0	114.8
24	35	65.6	73.1	1712	2597	77.3	79.1
25	41	94.5	72.1	2716	4257	76.8	132.4
26	41	134.4	70.4	4454	6375	76.6	198.4
27	48	165.9	71.2	5397	7602	77.0	239.0
28	48	131.6	70.9	4097	6317	77.8	190.7
29	62	161.6	70.5	4519	7578	77.0	238.8
30	62	170.5	69.5	5980	7625	76.5	259.7

* Indirect determination of DNAP.

**Ultraviolet determination.

Appendix III -- continued.

the right hind limb					
Myofibrillar protein N %	Sarcoplasmic protein N %	Connective tissue N %	% DNA* in dry muscle	% DNA** in dry muscle	Total N/ DNA*
25.8	48.0	6.6	1.67	1.57	6.3
25.8	48.0	6.6	1.67	1.57	6.0
23.7	52.1	6.7	1.75	1.63	6.1
23.7	52.1	6.7	1.75	1.63	6.1
20.2	54.8	5.9	1.64	1.55	6.9
20.2	54.8	5.9	1.64	1.55	6.1
25.9	51.3	5.7	1.51	1.40	7.0
27.5	49.7	6.1	1.49	1.37	7.8
29.2	47.1	6.5	1.42	1.32	7.9
32.6	46.0	5.7	1.53	1.39	7.8
31.9	47.0	6.3	1.24	1.14	9.8
42.5	35.5	5.1	1.13	1.05	9.6
36.3	43.2	6.5	1.20	1.08	8.8
43.6	38.8	5.7	0.74	0.66	15.4
47.1	33.4	6.4	0.63	0.58	19.7
51.0	32.7	4.3	0.51	0.46	25.1
51.1	33.7	3.1	0.54	0.49	23.7
51.1	32.5	4.6	0.49	0.46	26.9
52.1	32.3	3.6	0.30	0.32	39.7
54.4	30.7	4.5	0.36	0.33	37.2
55.9	28.9	4.0	0.33	0.30	40.9
55.1	28.2	6.1	0.30	0.28	44.9
58.6	27.5	3.0	-	-	-
52.5	30.7	5.9	0.35	0.32	38.3
56.2	28.7	3.5	0.31	0.29	43.2
54.8	31.8	3.8	0.28	0.25	47.5
55.4	28.3	5.8	-	-	-
54.4	31.3	3.6	0.26	0.24	52.3
54.2	30.8	4.4	0.25	0.24	54.8
58.6	28.9	3.2	0.22	0.20	65.9

APPENDIX IV

Body weight, muscle weight and chemical compositions of the left quadriceps muscles. Experiment II.

			Whole body			Quadriceps muscles		
Rat no.	Age day	Sex	Fresh weight gm	H_2O %	Total N gm	Fresh weight gm	H_2O %	Total N mg
Initial control								
1	25	M[1]	57.0	73.1	1.313	0.269	76.6	7.6
2	25	F[2]	55.1	73.8	1.224	0.230	78.3	6.6
3	27	M	47.6	75.5	1.171	0.202	76.4	6.4
4	27	F	55.2	73.7	1.367	0.278	77.9	8.1
Control								
1	30	M	74.8	74.4	1.997	0.332	77.4	10.3
2	30	F	69.6	74.2	1.758	0.331	76.7	11.6
3	37	M	106.9	74.5	2.683	0.581	77.5	17.1
4	37	F	105.7	74.7	2.791	0.632	76.6	19.2
5	43	M	152.9	73.4	4.204	0.833	76.6	26.8
6	43	F	124.8	73.7	3.550	0.772	77.5	25.1
7	50	M	155.5	69.7	5.289	0.998	77.1	32.1
8	50	F	152.5	72.0	4.477	0.984	77.3	31.8
9	56	M	187.0	73.3	5.794	1.345	75.2	46.6
10	55	F	160.0	71.0	4.462	1.014	76.1	34.7
11	96	M	236.0	69.8	7.269	1.555	75.7	54.2
12	96	M	352.0	69.3	11.852	2.569	75.5	88.9
13	98	M	262.0	67.1	7.863	1.727	75.0	63.9
14	98	M	340.0	68.2	11.005	2.210	75.4	80.5
15	97	F	158.0	68.7	4.917	1.120	75.3	37.6
16	98	F	205.0	66.9	6.069	1.522	75.5	53.8
17	99	F	223.0	66.1	6.626	1.553	75.4	56.7
18	99	F	240.5	68.8	7.493	1.782	75.0	61.8
Low plane								
1	30	M	68.0	75.9	1.426	0.324	77.5	9.8
2	30	F	56.2	76.3	1.158	0.261	77.0	8.0
3	37	M	80.8	73.4	1.535	0.380	75.6	12.0
4	37	F	67.4	75.1	1.368	0.380	76.9	11.7
5	42	M	75.9	74.9	1.734	0.439	76.0	14.5
6	42	F	77.9	74.8	1.613	0.380	76.1	12.4
7	50	M	71.0	74.8	1.680	0.394	76.8	13.3
8	50	F	71.0	75.1	1.641	0.377	77.3	11.7
9	56	M	84.0	74.1	1.869	0.477	77.0	15.3
10	54	M	80.0	74.9	1.696	0.462	77.2	14.6
11	56	F	76.0	74.3	1.869	0.397	76.4	13.3
12	54	F	81.0	73.2	1.898	0.523	75.9	17.4

Appendix IV -- continued.

13	103	M		129.0	72.1	3.99	0.843	76.4	28.9
14	101	M		100.5	72.7	2.475	0.684	76.9	23.3
15	102	M		157.5	72.0	3.912	1.200	76.0	43.2
16	102		F	108.5	73.5	2.515	0.809	76.6	27.4
17	104		F	113.5	71.2	2.816	0.780	75.8	27.4
18	104		F	118.7	71.8	3.553	0.960	75.5	34.1
19	102		F	113.7	71.2	2.687	0.848	75.3	30.0
Realimentation									
1	63	M		133.5	72.0	3.385	0.666	77.9	20.0
2	61		F	105.0	73.1	2.768	0.554	77.5	16.7
3	100	M		261.0	66.7	7.868	1.767	75.4	59.4
4	100	M		211.5	70.8	6.149	1.525	76.8	52.1
5	100	M		287.0	68.1	9.380	2.102	75.9	74.8
6	98	M		278.0	68.3	8.456	1.960	75.7	69.0
7	99	M		219.0	69.2	6.289	1.380	76.5	46.4
8	98	M		261.5	69.4	8.486	1.697	75.8	58.7
9	101		F	182.5	66.2	5.604	1.431	75.3	49.1
10	101		F	200.5	68.2	6.586	1.534	75.6	54.5
11	101		F	171.0	66.4	5.178	1.083	75.8	38.8
12	101		F	201.5	66.0	6.584	1.445	75.3	50.8
13	101		F	158.0	69.1	5.566	1.130	75.9	38.6
14	100		F	185.3	68.9	5.782	1.280	75.6	47.0
15	102		F	208.5	68.8	6.613	1.450	75.7	50.8

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[1] M, male

[2] F, female

APPENDIX V

Chemical compositions of the right hind limb muscles. Experiment II.

Rat no.	Fresh weight gm	H_2O %	Total N gm	Myofibrillar protein N gm	Total DNA*	Total DNA**	% DNA* in dry muscle	Total N/ total DNA*	Myofibrillar protein N/ Total DNA*
Initial control									
1	2.10	77.1	0.057	0.029	1776	1598	0.37	32.1	16.3
2	2.05	78.0	0.061	0.031	1575	1402	0.35	38.7	19.7
3	1.75	76.6	0.054	0.027	1599	1471	0.39	33.8	17.9
4	2.01	77.6	0.058	0.030	1530	1331	0.34	37.9	19.6
Control									
1	3.00	78.0	0.089	-	-	-	-	-	-
2	2.85	77.5	0.095	-	-	-	-	-	-
3	4.80	77.3	0.141	-	-	-	-	-	-
4	4.75	77.1	0.150	-	-	-	-	-	-
5	7.50	77.9	0.223	0.125	3395	3156	0.20	65.7	36.8
6	6.00	77.7	0.185	0.927	2750	2501	0.21	67.3	33.7
7	8.10	77.7	0.261	-	-	-	-	-	-
8	8.00	75.3	0.254	-	-	-	-	-	-
9	9.35	75.4	0.340	0.166	4291	3991	0.19	79.2	38.8
10	7.57	75.5	0.271	0.144	3823	3479	0.21	70.9	37.8
11	12.41	75.5	0.452	0.228	4700	4324	0.15	96.2	48.5
12	20.93	75.0	0.809	0.465	5102	4796	0.10	158.6	91.1
13	13.09	74.9	0.463	0.240	4412	3971	0.13	104.9	54.4
14	17.08	75.4	0.618	0.281	4803	4467	0.11	128.6	58.5
15	8.05	75.5	0.289	0.144	3411	3240	0.17	84.7	42.2
16	12.13	75.1	0.439	0.234	4252	3997	0.14	103.2	55.0
17	12.13	75.2	0.442	0.252	5812	5347	0.19	76.0	43.4
18	14.25	75.0	0.519	0.270	5013	4762	0.14	103.5	53.9
Low plane									
1	2.68	77.6	0.078	-	-	-	-	-	-
2	2.27	77.1	0.071	-	-	-	-	-	-
3	3.58	76.0	0.115	-	-	-	-	-	-
4	2.81	77.9	0.084	-	-	-	-	-	-
5	3.41	76.0	0.106	0.053	2614	2457	0.32	40.6	20.3
6	3.46	76.6	0.110	0.053	2287	2058	0.28	48.1	23.2
7	3.13	77.3	0.100	-	-	-	-	-	-
8	3.17	77.6	0.097	-	-	-	-	-	-
9	3.71	76.3	0.131	0.058	3004	2824	0.34	43.6	19.3
10	2.32	76.8	0.111	0.053	2442	2247	0.32	45.5	21.7
11	2.96	76.3	0.104	0.046	2421	2203	0.35	43.3	19.0
12	3.80	75.8	0.128	0.062	2416	2247	0.26	53.0	25.7
13	6.30	75.5	0.227	0.122	3192	2841	0.21	71.1	38.2
14	5.15	76.4	0.169	0.099	2904	2701	0.23	58.2	34.1
15	9.03	75.7	0.325	0.160	4307	3876	0.20	75.5	37.1
16	5.85	76.4	0.205	0.095	3592	3341	0.26	57.1	26.4
17	6.41	75.4	0.223	0.116	2603	2460	0.17	85.7	44.6
18	8.70	75.6	0.316	0.145	3111	2924	0.15	101.6	46.6
19	5.47	75.7	0.201	0.106	3140	2983	0.24	64.0	33.8

Appendix V -- continued.

Realimentation									
1	4.65	77.6	0.144	0.078	2611	2376	0.25	55.3	29.9
2	4.61	76.5	0.151	0.076	3014	2773	0.28	50.1	25.2
3	12.73	75.1	0.467	0.256	5015	4714	0.16	93.1	51.0
4	11.18	76.7	0.393	0.219	3792	3413	0.15	103.6	57.8
5	15.62	75.9	0.555	0.284	4607	4238	0.12	120.5	61.6
6	14.77	75.4	0.521	0.291	5611	5218	0.13	92.9	51.9
7	11.09	74.6	0.402	0.230	3404	3166	0.12	118.1	67.6
8	13.53	75.4	0.488	0.248	3450	3174	0.10	141.4	71.9
9	10.54	75.0	0.399	0.226	5381	4789	0.20	74.1	42.0
10	11.70	75.5	0.431	0.218	4212	4001	0.15	102.3	51.8
11	8.31	74.7	0.299	0.167	3903	3628	0.19	76.6	42.8
12	10.61	74.1	0.402	0.208	4412	4015	0.16	91.2	47.1
13	8.61	75.1	0.302	0.147	3704	3482	0.17	81.5	39.7
14	10.04	75.4	0.346	0.181	3296	2933	0.13	105.0	54.9
15	12.24	75.5	0.463	0.223	4007	3526	0.13	115.5	55.7

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* Indirect determination of DNAP.

**Ultraviolet determination.

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